The Herbal Medicine Sho-saiko-to Inhibits Growth and Metastasis of Malignant Melanoma Primarily Developed in *ret*-Transgenic Mice

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Sho-saiko-to is the most popular herbal medicine in Japan. We investigated the anti-tumor and anti-metastatic effects of Sho-saiko-to and its chemically defined ingredients on the primary skin melanoma that developed in a metallothionein-I (MT)/*ret* transgenic mouse line and on a melanoma cell line (Mel-ret), which was derived from a primary tumor developed in a MT/*ret* transgenic mouse. *In vitro,* Sho-saiko-to suppressed the growth of Mel-ret cells more strongly than any single ingredient of Sho-saiko-to, although baicalin as one of several ingredients tested also suppressed it significantly. *In vivo,* Sho-saiko-to (i) significantly ($p < 0.02$) prolonged the onset of tumor development (1.5 mo), (ii) definitely retarded the transition to malignancy, (iii) significantly decreased the incidence of distant metastasis to brain ($p < 0.002$), kidney ($p < 0.05$), and liver ($p < 0.05$) at the malignant stage, and (iv) significantly ($p < 0.02$) prolonged life span (2.6 mo). Moreover, Sho-saiko-to and baicalin down-regulated the matrix metalloproteinase-2 and −9 expression levels, and upregulated their inhibitor expression level in both the primary tumors and Mel-ret cells. In conclusion, Sho-saiko-to displayed anti-tumor and anti-metastatic effects on melanoma with regulation of the balance of matrix metalloproteinase and tissue inhibitor of the matrix metalloproteinase levels. *Key words: baicalin/Sho-saiko-to/matrix metalloproteinase. J Invest Dermatol 111: 640–644, 1998*

An effective protocol for the prevention and therapy of melanoma is urgently needed, because the recent record reduction in the Earth's ozone layer blocking the sun's ultraviolet rays may worsen the prediction made in 1987 that one in 90 Caucasians in the U.S.A. will develop skin melanoma by the turn of the century (Gleason *et al*, 1993).

Sho-saiko-to, which consists of seven crude ingredients extracted from herbs, has been widely used for chronic hepatitis and liver cirrhosis in Japan (Oka *et al*, 1995), but has yet to become popular in Western countries. Recently, Yano *et al* (1994) showed that Sho-saiko-to suppresses the proliferation of hepatocellular carcinoma cell lines *in vitro* by inducing apoptosis and arrest at the G_0/G_1 phase; however, there are no reports regarding whether Sho-saiko-to has an effect on primary melanoma development, the shift to malignant stage (melanoma) from benign stage (melanocytic nevus), and the metastasis of primary melanoma, mainly because no earlier animal models were suitable to test these points.

Previously, we have succeeded in establishing a transgenic mouse line in which melanocytic tumors primarily develop, by introducing the *ret* oncogene fused to the mouse metallothionein-I (MT) promoter-enhancer (Iwamoto *et al*, 1991). The pathologic changes in MT/*ret* transgenic mice developed stepwise as the mice aged. Recently, we have found that the melanocytic tumor that developed in MT/*ret* transgenic mice of line 304 finally progressed to melanoma accompanying distant metastasis (Kato *et al*, 1998). In this transgenic mouse line, tumors developed mainly in the skin, where we could inspect them throughout life. For these reasons, this transgenic mouse line appeared especially suitable for a long-term stage-oriented examination of both the anti-tumor and the anti-metastatic effects of drugs.

In this study, we demonstrate for the first time that Sho-saiko-to suppresses primary tumor development, transition from the benign to the malignant stage, and tumor growth and distant metastasis, resulting in a prolongation of life span, in the transgenic mouse model in which melanoma spontaneously develops. Furthermore, concerning its mechanism of anti-tumor and anti-metastatic effects, evidence is provided that Sho-saiko-to controls the cell cycle and expression levels of matrix metalloproteinase (MMP) and the tissue inhibitor of matrix metalloproteinase (TIMP) of tumor cells.

MATERIALS AND METHODS

Sho-saiko-to treatment in MT/*ret* transgenic mice The MT/*ret* transgenic mice had no tumors for 4.8 mo on average after birth (tumor-free stage). Next, benign melanocytic tumors in the skin developed without exception and grew slowly, while the tumor was histologically benign (benign stage; corresponding with **Fig 3*C*, *D***). Then, tumors that developed in the skin of these mice suddenly began to grow rapidly with ulcers on the surface, and displayed the histologic appearance of typical melanoma (malignant stage; corresponding with **Fig 3*A*, *B***) (Iwamoto *et al*, 1991; Kato *et al*, 1998). MT/*ret* transgenic mice were randomly divided into two groups. One group (experimental, n = 94) was given drinking water containing 3–5 mg Sho-saiko-to (TUMURA, Tokyo, Japan) per d per mouse, which is the usual

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Abbreviations: MMP, matrix metalloproteinase; MT, metallothionein-I; TIMP, tissue inhibitor of the matrix metalloproteinase.

human dose in conversion per weight, from 1 mo of age until death. The other group (control, n = 90) was given plain drinking water only. Each of the two groups consisted of several evenly divided litters. For analysis of tumor volume and life span, 50 transgenic mice in each group were used. For examination of distant metastasis and/or MMP and TIMP expression levels in primary tumors, 30 (control) or 34 (experimental) transgenic mice were tested.

Analysis of metastasis of primary tumors in MT/*ret* transgenic mice Metastasis of primary tumor in 9–15 mo old litter mice of experimental and control groups was analyzed on hematoxylin and eosin stained sequential specimens of primary tumors, brain, kidney, liver, spleen, lung, and lymph nodes.

Cell line and culture Mel-ret cells, which had been established from the tumor of a MT/*ret* transgenic mouse of line 304 as a melanoma cell line (Taniguchi *et al*, 1992), were suspended in an RPMI-1640 medium supplemented with 10% fetal calf serum and 100 U penicillin per ml, 100 μg streptomycin per ml, 2 mM L-glutamine, and 50 μM 2-mercaptoethanol (complete medium) in 60 × 15 mm tissue culture dishes (Coming, NY). The water soluble reagent, Sho-saiko-to, was dissolved in the complete medium to a concentration of 10 mg per ml. This solution was centrifuged (1200 × *g*, 20 min) to remove insoluble ingredients, and the supernatant was sequentially passed through 0.22 μm filters for sterilization. The known representative chemical ingredients of Sho-saiko-to, such as baicalin, glycyrrhizin, baicalein, saikosaponin-d, and ginsenoside Rb1, were obtained from Wako Pure Chemical Industries (Osaka, Japan). Each of these ingredients of Sho-saiko-to was first dissolved in dimethylsulfoxide (Wako Pure Chemical Industries), and diluted in complete medium to 0.05% or lower concentration as described elsewhere (Yano *et al*, 1994) because it was not water soluble. Corresponding to the earlier report of Yano *et al* (1994) that ≤2000 μg Sho-saiko-to per ml had no influence on the viability of normal human peripheral lymphocytes and normal rat hepatocytes within 3 d of culture, Sho-saiko-to at the concentration of 400 μg per ml and the amount of each ingredient (baicalin, baicalein, glycyrrhizin, saikosaponin-d, and ginsenoside Rb1) contained in 400 μg Sho-saiko-to per ml displayed no nonspecific cytotoxicity on normal mouse splenic lymphocytes *in vitro*, either when cell viability was examined after 24 h incubation by the dye exclusion test or when growth response to mitogen (concanavalin A) was measured by [^{3}H]thymidine uptake. In human, after oral treatment of clinically applicable amounts of Sho-saiko-to, the blood concentration of baicalein roughly reached the level of baicalein contained in 200 μg Sho-saiko-to per ml (Nishioka *et al*, 1992). Therefore, 400 μg Sho-saiko-to per ml is not too high a concentration for practical use. DNA synthesis of Mel-ret cells in the presence or absence of Sho-saiko-to or its ingredients was measured by [^{3}H]thymidine uptake as described previously (Kato *et al*, 1994).

Analysis of cell cycle Tumor cells prepared from the Sho-saiko-to treated and untreated MT/*ret* transgenic mice were analyzed for cell cycle by laser flow-cytometry using propidium iodide as a dye staining DNA, according to the previously described method (Hayakawa *et al*, 1994).

Analysis of MMP-2, MMP-9, and TIMP-2 expression The immunoblotting assay of MMP-2, MMP-9, and TIMP-2 proteins was done by the method described earlier (Pu *et al*, 1996). Fixed amounts (50 μg per lane) of total cell lysates prepared from tumors developed in MT/*ret* transgenic mice or Mel-ret-cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Nihon Millipore Kogyo KK, Yonezawa, Japan). The membranes were incubated overnight with the anti-MMP-2 polyclonal antibody, which was prepared by immunizing rabbits with purified MMP-2 from murine colon carcinoma cells, anti-MMP-9 monoclonal antibody (9AG) (Sakata *et al*, 1996), or anti-TIMP-2 polyclonal antibody (Chemicon International, CA), followed by incubation with the second antibody (goat anti-rabbit or rat IgG conjugated to horseradish peroxidase; Tago, Burlingame, CA) for 1.2 h. The proteins were visualized by western blot chemiluminescence reagent (DuPont NEN, Boston, MA) as directed by the manufacturer.

Statistical analysis Analysis of tumor volume and life span in the Sho-saiko-to treated and control mice was done by the Mann–Whitney U test. Analysis of the percentage of metastasis in the Sho-saiko-to treated and untreated control mice was done by chi-square (χ^2) test or Fisher's exact test.

RESULTS

Sho-saiko-to inhibits growth of primary melanocytic tumors in MT/*ret* transgenic mice As shown in **Fig 1**, we first examined the effect of Sho-saiko-to and its ingredients on the growth of Mel-ret cells *in vitro*. Sho-saiko-to ($p < 0.005$) and one of its main ingredients, baicalin ($p < 0.02$), significantly inhibited DNA synthesis of Mel-ret cells, compared with control; and the level of DNA synthesis of Mel-ret cells treated with Sho-saiko-to was significantly lower ($p < 0.005$) than that of cells treated with any single ingredient including baicalin. Following this basic observation, which corresponds to the earlier result of Yano *et al* (1994) for human cancer cells, we examined the effect of Sho-saiko-to on the growth of primary tumors in MT/*ret* transgenic mice. As shown in **Fig 2**, Sho-saiko-to displayed anti-tumor effects at different stages of tumor growth. First, the duration of the tumor-free stage in Sho-saiko-to treated transgenic mice (6.3 ± 2.6 mo, n = 50) was significantly longer ($p < 0.02$) than that of untreated mice (4.8 ± 2.6 mo, n = 50). Second, Sho-saiko-to remarkably lowered the rate of growth of tumors after their initial development. Whereas primary tumors quickly shifted from <150 mm^3 (3.3 mo after birth on average) to >5000 mm^3 (7.5 mo after birth on average) in Sho-saiko-to untreated control mice, those rates remained at <150 mm^3 (9.0 mo after birth on average) for a long time in Sho-saiko-to treated mice. Histologic examination of some tumors from Sho-saiko-to treated or untreated control mice confirmed that tumors with a volume of <150 mm^3 are at the benign stage, whereas those with a volume of >5000 mm^3 are at the malignant stage. In the photographs of representative Sho-saiko-to treated and untreated control 10 mo old transgenic mice of the same litter, a tumor in a treated mouse was kept at the benign stage (**Fig 3*C*, *D***), whereas that in an untreated mouse reached the malignant stage (**Fig 3*A*, *B***). These results suggest that Sho-saiko-to delays both the onset of primary tumor development and the shift from benign stage to malignant stage of the tumor. During the malignant stage, Sho-saiko-to still inhibited tumor growth significantly, though less markedly than during the benign stage.

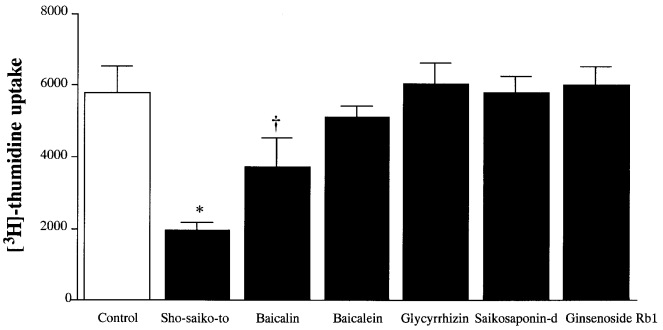

Figure 1. Sho-saiko-to inhibits the growth of melanoma cells *in vitro*. Sho-saiko-to (400 μg per ml) or each of its main ingredients was added on day 1 of *in vitro* culture of Mel-ret cells. Their DNA synthesis on day 3 was measured by [^{3}H]thymidine uptake assay. Each column shows the mean ± SD of six cultures. Shown is a representative of four experiments. *,†Significantly different (*$p < 0.005$; †$p < 0.02$) from the control by the Mann–Whitney U test.

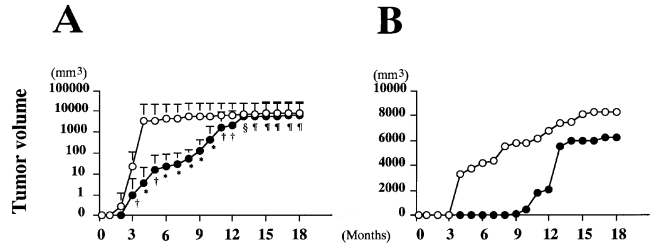

Figure 2. Sho-saiko-to inhibits primary development and growth of melanocytic tumors in MT/*ret* transgenic mice. Tumor volumes (mean ± SD) are shown for Sho-saiko-to treated (●, n = 50) and untreated control (○, n = 50) transgenic mice on the log (*A*) and linear (*B*) scales. *,†,§,¶Significantly different (*$p < 0.001$; †$p < 0.005$; §$p < 0.01$; ¶ $p < 0.05$) from the untreated control by the Mann–Whitney U test. Tumor volumes in mice that died before 18 mo were calculated as a continuation of the volume up to 18 mo.

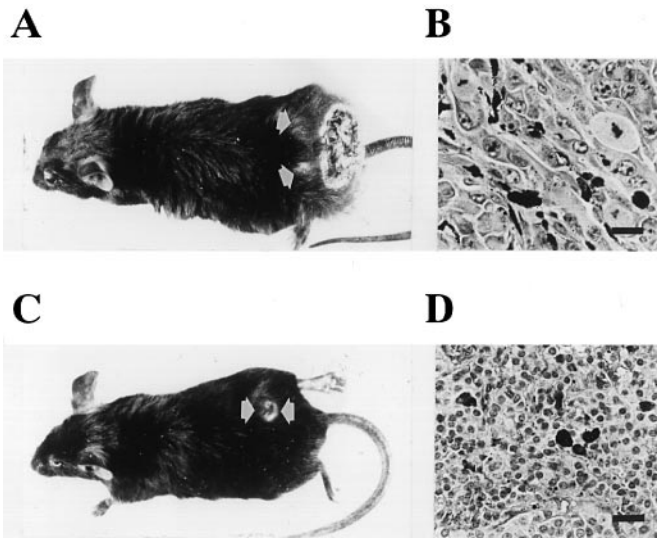

Figure 3. Representative 10 mo old MT/*ret* transgenic mice of the same litter treated or untreated with Sho-saiko-to. Macroscopic (*A*, *C*) and microscopic (*B*, *D*; hematoxylin and eosin staining) appearance of the control (*A*, *B*) and experimental (*C*, *D*) transgenic mice were displayed. They all exhibit black coat color (*A*, *C*). Note the severe systemic skin melanosis and black tumors (*A*, 40 × 35 × 30 mm; *C*, 6 × 6 × 3 mm; indicated by *arrows*). Tumors frequently developed in back skin as shown in this picture, but they also developed in other areas of skin. In the control mouse, the tumor consisted of atypical cells bearing variable sizes of nuclei with high mitotic activity (malignant feature) (*B*; hematoxylin and eosin staining). In the experimental mouse (*D*), the tumor consisted of round cells bearing round nuclei without mitotic activity (benign feature). *Scale bar*: 15 μm.

Table I. Sho-saiko-to partially inhibits metastasis of primary melanocytic tumors at the malignant stage[a]

	Incidence of metastasis	
Site of metastasis	Control	Sho-saiko-to treated
Brain	46.7%	11.8%*
Kidney	13.3%	0%†
Liver	13.3%	0%†
Spleen	6.7%	0%
Lung	46.7%	41.2%
Lymph node	53.3%	52.9%

[a]Sho-saiko-to treated (n = 34) and untreated (n = 30) MT/*ret* transgenic mice that bear tumors at the malignant stage were sacrificed for examination of metastasis of tumors. *,†Significantly different values (*$p < 0.002$; †$p < 0.05$) from those for the control group by chi-square test (brain, lung, and lymph node) or Fisher's exact test (kidney, liver, and spleen).

Sho-saiko-to inhibits metastasis of primary melanocytic tumors at the malignant stage We next examined the effect of Sho-saiko-to on distant metastasis in MT/*ret* transgenic mice. The incidences (%) of distant metastasis for brain ($p < 0.002$), kidney ($p < 0.05$), and liver ($p < 0.05$) in the transgenic mice given Sho-saiko-to (n = 34) were significantly lower than those in the controls (n = 30), when mice bearing malignant stage tumors of equivalent volumes (**Table I**) and those from littermate mice (data not shown) were examined. There was little or no difference, however, between the experimental and control groups in the incidence of distant metastasis for spleen, lung, and lymph node. These results suggest that Sho-saiko-to partially inhibits the distant metastasis of melanoma at the malignant stage.

Sho-saiko-to prolongs the life span of MT/*ret* transgenic mice We further studied the effect of Sho-saiko-to on the life span of MT/*ret* transgenic mice. **Figure 4** shows the survival time and rate of Sho-saiko-to treated (n = 50) and untreated (n = 50) MT/*ret* transgenic mice. Mean life span of the former (12.6 ± 5.0 mo) was significantly ($p < 0.02$) longer than that of the latter (10.0 ± 4.3 mo).

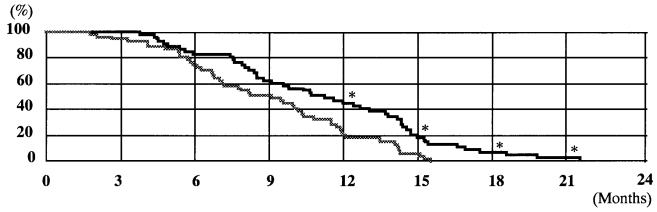

Figure 4. Sho-saiko-to prolongs the life span of MT/*ret* transgenic mice. Survival rates of Sho-saiko-to treated (■; n = 50) and untreated control (▨; n = 50) transgenic mice. *Significantly different ($p < 0.02$) from the untreated control by the Mann–Whitney U test.

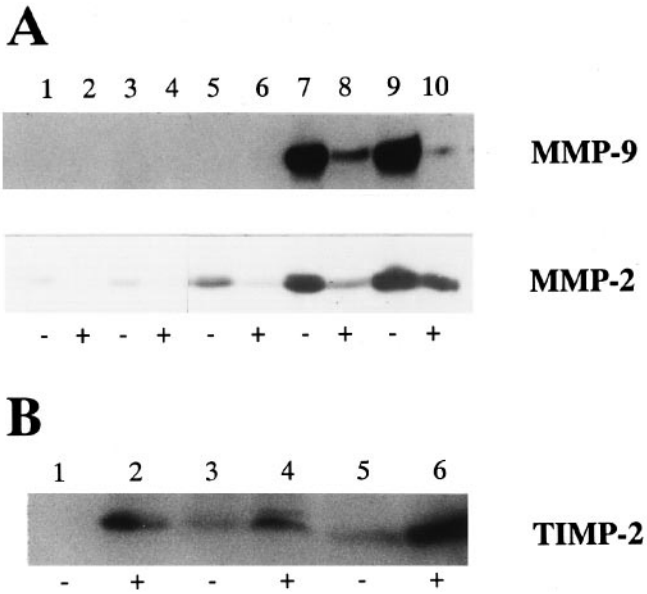

Figure 5. Sho-saiko-to regulates the MMP-2, MMP-9, and TIMP-2 expression levels in primary melanocytic tumors in MT/*ret* transgenic mice. (*A*) The MMP-2 and MMP-9 protein expression levels are shown in the primary tumors with different volumes (*lane 1*, 100 mm^3; *lane 2*, 112 mm^3; *lane 3*, 2250 mm^3; *lane 4*, 2704 mm^3; *lane 5*, 13,750 mm^3; *lane 6*, 13,750 mm^3; *lane 7*, 26,250 mm^3; *lane 8*, 32,000 mm^3; *lane 9*, 102,250 mm^3; *lane 10*, 120,000 mm^3) developed in Sho-saiko-to treated (+) (*lanes 2*, *4*, *6*, *8*, and *10*) and untreated control (–) (*lanes 1*, *3*, *5*, *7*, and *9*) MT/*ret* transgenic mice. The tumors for *lanes 2* and *9*, and those for *lanes 4* and *7* were taken from the same litter MT/*ret* transgenic mice. (*B*) The TIMP-2 protein expression levels are shown in the primary tumors with different volumes (*lane 1*, 97 mm^3; *lane 2*, 120 mm^3; *lane 3*, 2450 mm^3; *lane 4*, 2670 mm^3; *lane 5*, 20,000 mm^3; *lane 6*, 20,000 mm^3) developed in Sho-saiko-to treated (+) (*lanes 2*, *4*, and *6*) and untreated control (–) (*lanes 1*, *3*, and *5*) mice. Similar results were also obtained with tumors from other control (n = 15) and experimental (n = 15) mice.

Survival rate of the former was also significantly ($p < 0.02$) higher than that of the latter from 12 mo after birth.

Sho-saiko-to arrests cell cycle of primary melanocytic tumors at the G_0/G_1 phase The percentage of the G_0/G_1 phase of the tumor cells (mean ± SD) from the experimental mice (G_0/G_1, 86.7 ± 1.4%; S, 3.0 ± 1.1%; $G_{2/M}$, 10.3 ± 0.7%, n = 4) was significantly higher ($p < 0.01$) than that from the control mice (G_0/G_1, 75.9 ± 3.1%; S, 6.9 ± 2.7%; $G_{2/M}$, 17.2 ± 2.6%, n = 4) when we examined the histologically determined benign stage tumors of equivalent volumes. These results suggest that Sho-saiko-to suppresses the tumor growth through cell cycle arrest at the G_0/G_1 phase *in vivo*.

Sho-saiko-to changes the levels of MMP and TIMP-2 in melanocytic tumors In order to partially determine the mechanism of the anti-tumor and anti-metastatic effects of Sho-saiko-to, we compared the levels of MMP and TIMP-2 expression in tumors from the Sho-saiko-to treated and untreated mice. In tumors of control MT/*ret* transgenic mice, the protein expression levels of MMP-9 and MMP-2 remarkably increased when tumor size reached >20,000 mm^3 (fully

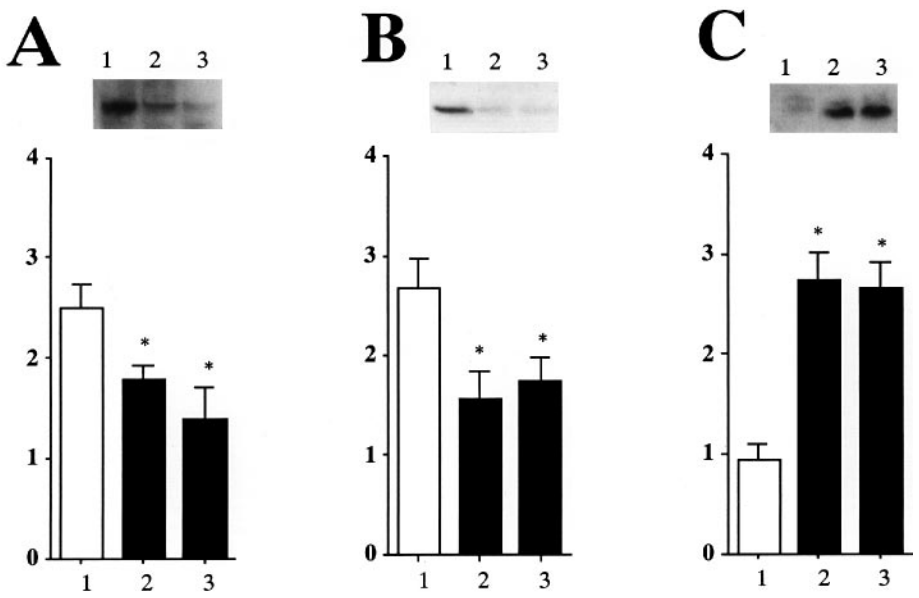

Figure 6. Sho-saiko-to and balcalin regulate the MMP-2, MMP-9, and TIMP-2 expression levels in melanoma cells *in vitro*. Mel-ret cells were cultured for 3 d in the absence (*lane 1*) or presence of 400 μg Sho-saiko-to per ml (*lane 2*) or baicalin contained in 400 μg Sho-saiko-to per ml (*lane 3*) for measurement of MMP-9 (*A*), MMP-2 (*B*), and TIMP-2 (*C*) protein expression levels. Upper panels show representative pictures from four experiments, and the result of statistical analysis of data (n = 4) measured by densitometry for the bands are shown in the lower panels. Each column shows the mean ± SD. *Significantly different ($p < 0.05$) from the control (□) by the Mann–Whitney U test.

malignant stage) (*lanes 7* and *9* in **Fig 5*A***). No such increase was seen in tumors from age-matched Sho-saiko-to treated MT/*ret* transgenic mice (*lane 2 versus lane 9* as control; *lane 4 versus lane 7* as control in **Fig 5*A***). Furthermore, the increase in the MMP-2 and MMP-9 expression levels in tumors from Sho-saiko-to treated mice was not so remarkable as that in tumors from untreated control mice, even when compared with size-matched tumors from mice of different ages (*lane 8 versus lane 7* as control; *lane 10 versus lane 9* as control in **Fig 5*A***). In contrast, as compared with the levels in size-matched tumors from the untreated control mice, the levels of TIMP-2 expression markedly increased in both small (*lane 2 versus lane 1* as control; *lane 4 versus lane 3* as control) and large (*lane 6 versus lane 5* as control) stages of tumors from the Sho-saiko-to treated mice (**Fig 5*B***).

Finally, we examined whether the regulation of MMP and TIMP levels by Sho-saiko-to *in vivo* would hold for Mel-ret cells *in vitro*, and whether baicalin plays a crucial role in this regulation. There was little difference in the levels of MMP-2, MMP-9, and TIMP-2 expression between Sho-saiko-to or baicalin treated cells and untreated control cells on days 1 and 2 of culture (data not shown). As shown in **Fig 6**, however, both Sho-saiko-to and baicalin downregulated the expression of MMP-2 and MMP-9, and upregulated the expression of TIMP-2 on day 3 of culture.

DISCUSSION

We have demonstrated that Sho-saiko-to delays both the onset of primary tumor (melanoma) development and the shift from benign stage to malignant stage, which results in more than 25% prolongation of the life span of tumors developing transgenic mice. The anti-tumor and anti-metastatic effects of this herbal medicine partially protected animals from primarily developing tumors. Any earlier reports did not examine the long-term effects of the medicine on individual animals ranging from the early life before tumor development to the end stage of tumor death. In this study, we have provided evidence that Sho-saiko-to inhibits proliferation of tumor cells through cell cycle arrest at the G_0/G_1 phase *in vivo*. One of the mechanisms of the anti-tumor effects of this medicine against melanoma development in transgenic mice should therefore be the regulation of cell cycle, as suggested previously by an *in vitro* experiment with carcinoma cells (Yano *et al*, 1994).

Sho-saiko-to has been further shown to partially inhibit metastasis to distant tissues at the malignant stage. Correspondingly, the levels of MMP-9 and MMP-2 in tumors at the malignant stage from the Sho-saiko-to treated mice were lower than those in tumors with equivalent volumes from untreated control mice. In contrast, the tumors from the Sho-saiko-to treated mice had higher levels of TIMP-2 as an inhibitor of MMP than those with an equivalent volume from control mice. MMP are thought to be associated with remodeling in many normal and pathologic processes, and in particular to be critical for metastasis (Crawford and Matrisian, 1994; Himelstein *et al*, 1994). Recent studies of human tissues or tumor-derived cell lines have also suggested the importance of dysregulation of the balance of MMP and TIMP in tumor progression including metastasis (Himelstein *et al*, 1994; Montgomery *et al*, 1994). Sho-saiko-to could therefore inhibit metastasis of melanoma in MT/*ret* transgenic mice through regulating the balance of MMP and TIMP levels.

TIMP may also regulate cell growth in a manner that is independent of their ability to inhibit MMP activity (Bertaux *et al*, 1991; Hayakawa *et al*, 1992; Stetler-Stevenson *et al*, 1992; Nemeth and Goolsby, 1993). By transfection of complementary DNA-encoding human TIMP-2, the growth of a human melanoma cell line (M24net cells) was markedly reduced in the skin of immunodeficient mice (Montgomery *et al*, 1994). Our result that the level of TIMP-2 was upregulated in small and medium-size tumors that developed in MT/*ret* transgenic mice and Mel-ret cells by treatment with Sho-saiko-to, thereby suggests that this upregulation may underlie the mechanism of tumor growth inhibition and cell cycle arrest by Sho-saiko-to.

A question arises whether the demonstrated anti-tumor and anti-metastatic effect is through regulation of the expression of the *ret* oncogene. Our preliminary study, however, showed that Sho-saiko-to treatment did not attenuate the expression of the *ret* oncogene in tumors from MT/*ret* transgenic mice and Mel-ret cells (Kato *et al*, unpublished observation). This may suggest that Sho-saiko-to affects regulatory elements of tumor progression other than the oncogene (*ret*) expression.

It is known that Sho-saiko-to consists of baicalin, baicalein, glycyrrhizin, saikosaponin, and ginsenoside as chemically defined ingredients (Zhang *et al*, 1993, 1995; Kato *et al*, 1994, 1995; Yano *et al*, 1994). Our study demonstrated that, among these Sho-saiko-to ingredients, baicalin was most potent in inhibiting the growth of Mel-ret cells, and in changing the levels of MMP-2, MMP-9, and TIMP-2 expression in the cells. Baicalin must therefore be one of the major ingredients of Sho-saiko-to that is active for the anti-tumor effect regulating the balance of MMP and TIMP levels; however, this one ingredient probably accounts for only a portion of the herb's entire effect on various anti-tumor parameters *in vivo*. Potentially cooperative roles of other ingredients in the overall anti-tumor effect of Sho-saiko-to *in vivo* remain to be elucidated.

In short, we showed that Sho-saiko-to displays anti-tumor and anti-metastatic effects on melanoma at least in part through the regulation of MMP and TIMP levels in tumor cells, and that baicalin could be one of the major ingredients of Sho-saiko-to responsible for these effects. Because the known clinical side-effects of long-term usage of

Sho-saiko-to are limited compared with those of other anti-turnor drugs (Ito *et al*, 1982; Sameshima *et al*, 1987), this herbal medicine might be useful for suppressing the overall process of the progression of melanoma in human.

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